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EXAMINER

LIU, SAMUEL W

ART UNIT PAPER NUMBER

1653

DATE MAILED: 11/05/2002

14

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary

Application No.

09/848,780

Applicant(s)

SANDERS, MITCHELL C.

Examiner

Samuel W Liu

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-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on ____.
- 2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 1-5 is/are pending in the application.
- 4a) Of the above claim(s) none is/are withdrawn from consideration.
- 5) ☐ Claim(s) ____ is/are allowed.
- 6) ☒ Claim(s) 1-5 is/are rejected.
- 7) ☐ Claim(s) ____ is/are objected to.
- 8) ☐ Claim(s) ____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☒ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on ____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
- Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
- 11) ☐ The proposed drawing correction filed on ____ is: a) ☐ approved b) ☐ disapproved by the Examiner.
- If approved, corrected drawings are required in reply to this Office action.
- 12) ☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

- 13) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. ____.
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).
- * See the attached detailed Office action for a list of the certified copies not received.
- 14) ☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).
- a) ☐ The translation of the foreign language provisional application has been received.
- 15) ☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

- 1) ☒ Notice of References Cited (PTO-892) 4) ☐ Interview Summary (PTO-413) Paper No(s). ____
- 2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948) 5) ☐ Notice of Informal Patent Application (PTO-152)
- 3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) 10 & 13. 6) ☐ Other: .

DETAILED ACTION

Applicants' claim for priority under 35 U.S.C. 119 (e) in the declaration with respect to Application No. 60/201407 is acknowledged. Preliminary amendment filed 8 January 2002 (Paper No. 8) prior to patent examination as to amendment of the specification has been entered.

Claims 1-5 are pending and examined in this Office action.

Claims/Specification Objections

The disclosure is objected to because of the following informalities:

- (1) In Claim 1, "wildtype" is suggested to be changed to "wild-type" in order to be consistent with the recitation in the specification (see page 7, line 4). See also abstract (line 4).
 - (2) In page 3, line 16, "CNBr" should be spelled out in full at the first instance of use; see also page 4, line 16, "SDS PAGE"; Page 4, line 21, "pipes" [suggest Piperazine-1,4-bis(2-ethanesulphonic acid)]; and page 9, line 22, "RPM"; and page 9, line 24 "PMSF".
 - (3) In page 5, line 20, "37-C" should be changed to "37 °C".
 - (4) In abstract, the term "pROTECT" should be appropriately spelled out.
- Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter that the applicant regards as his invention.

Claims 1-5 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

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The abbreviation in claims 1-5 should be fully spelled out at first instance of use. For clarity please change OD (claim 1), IPTG (claim 1), Qcolumn (claim 3), Macroprep ()column Claims 1-5 (Trade name, claim 3), His-tagged (claim 4), Ni-NTA (claim 4), and BSA to the full spelled out words.

Claims should begin with a capital letter and end with a period. Thus, the “a.” *etc.* in claims should for clarity be changed to “(a) *etc.* to eliminate this issue.

Claims 1-5 contain extra periods “vector.” (claim 1, item a), “domain.” (claim 1, item b)., “cells.” (claim 1 item c). See also claims 2-5. In addition, claims 1 and 3 do not end with a period.

Claim 2 recites “inserting said vectors into bacterial cells”; the recitation of vectors lacks antecedent basis in “a vector” (claims, line 3 and/or 4).

Claim 3 is indefinite in the recitation “Q column”; what is Q column? (note Q column can be refers to Q Sepharose fast flow column, Hitrap Q Sepharose column, Q Sepharose XL column, and Mono Q FPLC column (Pharmacia) *etc.*; and different Q type columns have different sample capacity, different pH -stability, and distinct /different elution profiles. In addition, claim 3 is unclear as to the recitation “Macroprep ()column”; what does “()column” regard? Note that MacroPrep[®] is a trade name. Where a trademark or trade name is used in a claim as a limitation to identify or describe a particular material or product, the claim does not comply with the requirements of 35 U.S.C. 112, second paragraph. See *Ex parte Simpson*, 218 USPQ 1020 (Bd. App. 1982). The claim scope is uncertain since the trademark or trade name

cannot be used properly to identify any particular material or product. A trademark or trade name is used to identify a source of goods, and not the goods themselves. Thus, a trademark or trade name does not identify or describe the goods associated with the trademark or trade name. In the present case, the trademark/trade name is used to identify/describe the matrix material used in the claimed process and, accordingly, the identification/description is indefinite. Also, the recitation “separating the protein in 100 mM Glycine pH 2.5” (item [d] of the claim) is unclear as to (i) whether or not the 100 mM Glycine solution is utilized for loading sample prior to applying the sample to the column or employed as a running buffer for the chromatography.

Claim 4 is rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. The omitted step is: apply the supernatant resulted from the centrifugation to the chromatographic column because without this step nowhere in the claim indicates what will be run on the column. Claim 4 recites “centrifuging out cell debris”; the recitation is not apparent as to whether or not the supernatant from the centrifugation step is applied to the chromatographic column or further is processed.

Claim 5 recites “coupling”; the recitation is not apparent regarding whether or not “coupling” refers to covalent conjugation or non-covalent attachment of the purified bovine α -crystallin protein to a chromatographic resin, *e.g.* via chelating mechanism. In addition, claim 5 is unclear as to the recitation (item a)) “a chromatography resin. CNBr-activated Sepharose 4B NHS-activated Sepharose 4B”; it appears that “or” is missing between the recitations “CNBr-activated Sepharose 4B” and “NHS-activated Sepharose 4B” because “a ... resin” is recited in precedence. Also, claim 5 is unclear in the recitation “rinsing and blocking said resin with BSA”;

does the recitation refer to using a pure BSA (*e.g.* 100% BSA solution in water) or a buffer solution containing BAS? Further, “blocking said resin...” renders claim 5 vague with respect to whether or not “blocking” refers to non-specific saturation of the resins or specifically “blocking” the binding sites for the protein to be separated. Note that claim 5 is also indefinite due to the extra period “.” in “resin” and “BSA .”.

Claim Rejections - 35 USC §103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the

Claim 1 is rejected under 35 U.S.C. 103(a) as being obvious over Liang, P. *et al.* (*Dev. Biol.* (1999) 207, 445-456) taken with Pilon, A. L. *et al.* (*Biotechnol. Prog.* (1996)12, 331-337) and Wittliff, J. L. *et al.* (*J. Biol. Chem.* (1990) 265, 22016-22022).

Liang *et al.* demonstrate that p26, a small heat shock/ α -crystallin protein, has chaperone activity *in vivo* and protracts the proteins from stress-induced denaturation (see the last two lines of Abstract), and teach the molecular chaperone proteins function of facilitating the folding of nascent protein, preventing aggregation when misfolded, and assisting with their distribute in cells (see the left column of page 446); *i.e.*, the chaperone proteins, in general, promote

production of soluble and active forms of target protein. Yet, Liang *et al.* do not expressly teach construction of recombinant fusion protein wherein p26 polypeptide and a polypeptide on which p26 works are in a “*cis*” manner in the fusion construct.

Pilon *et al.* teach a chaperon protein, ubiquitin (Ub) (see the last sentence of the first paragraph at page 331 and the bridging columns at page 335), functions facilitating Ub-fusion protein expression at high levels in soluble and stable form (see the right column at page 331 and figures 4-6), even soluble fraction reaches 100% (see the second paragraph of the right column at page 333). Also, Pilon *et al.* teach method of construction of the chaperone-(target) protein fusions, bacterial growth condition for the fusion protein expression upon IPTG induction (see “Experimental protocols” section).

Wittliff *et al.* teach that Ub-estrogen receptor expressed in *E.coli* retains characteristics of the native protein found in human tissue, suggesting the “*cis*” chaperone fusion generate not only soluble but active form of the interest protein (see abstract and Figures 3-4).

One of ordinary skill in the art would have combined the teachings of Liang *et al.* together with Pilon *et al.* and Wittliff *et al.* for the following advantages: (a) since p26 has chaperone activity *in vivo*, *e.g.* during living organism development, as suggested by Liang *et al.*, p26-protein (target) fusion would have been explored onto a therapeutic use apart from *in vitro* applications; and (b) the chaperone-protein (target) fusion are highly soluble and biologically active compared to native protein in *E.coli*.; this approach provides an avenue to make and use human steroid hormone receptor as well as other transcriptional factors *etc.* which are of great interest in pharmaceuticals but previously had proven difficult to ascertain as taught by Wittliff *et al.* (see the last paragraph of Introduction section at page 22017).

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Given the above motivation one of ordinary skill in the art would have combined the teachings of Liang *et al.*, Pilon *et al.* and Wittliff *et al.* with respect to development of a method of producing soluble and active recombinant p26-protein (target) wherein p26 is fused to the down stream protein of choice in a bacterial strain. Thus, the claimed invention was *prima facie* obvious to make and use at the time it was made.

Claim 2 is rejected under 35 U.S.C. 103(a) as being obvious over Wangner, B. J. *et al.* (*Ach. Biochem. Biophys.* (1995) 323, 455-462) taken with Julianus, G. *et al.* (US Pat. No. 5804417) and Adams, J. (*Cancer Res.* (1999) 59, 2615-2622).

Julianus *et al.* teach co-expression of a chaperone protein and a desired bioactive protein which allows for the correct folding and preventing aggregation of protein (see especially claim 1 and summary of the invention, columns 2-3). Also, Julianus *et al.* teach co-transformation of the vector containing the chaperon protein and the vector having the desired protein into a bacterial expression strain and the growth condition for the expression (see example 1, columns 13-14 and claim 1), as applied to items a) to e) of claim 2.

One of ordinary skill in the art would have combined the teachings of Wangner *et al.* and Julianus *et al.* to develop a method for preventing proteolysis of a recombinant protein expressed in bacteria. When combined, it would result in the following advantages:

bovine α -crystallin not only acts a molecular chaperone to help protein folding and/or refolding when underwent misfolding, and to prevent protein aggregation as taught by Julianus *et al.* (see lines 45-57, column 12), but also acts as a proteinase inhibitor – inhibiting multicatalytic proteinase (proteasome) activity as taught by Wagner *et al.* reference (see abstract, lines 16-19, and Figure 8 data). Note that proteasome is proteolytic complexes composed of

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peptidase/proteinase that degrade cytosolic and nuclear proteins, highly conserved from yeast to human being one of most abundant proteins in mammals; and proteasome regulates process that plays major roles in a variety of basic pathways during cell life and death as well as in health and disease/disorder states, *e.g.* cancer (see the Adams *et al.* reference). Therefore, co-expression of α -crystallin chaperone and desired protein in the same host cell, *e.g.* *E.coli.* would have been obvious to the skilled artisan in order to produce active and soluble recombinant proteins with high yield. The skilled artisan would also have been motivated to take the co-expression of the bovine α -crystallin and a desired protein in bacteria as a the first step so as to explore the avenue with respect to development of the α -crystallin as *proteasome inhibitor* for use in therapeutics, *e.g.* anticancer therapy (see Adams *et al.* reference) *via* co-transfection/co-infection of mammalian cells.

Claim 3 is rejected under 35 U.S.C. 103(a) as being obvious over Gopalakrishnan, S. *et al.* (*Invest. Ophthal. Vis. Sci.* (1992) 33, 2936-2941) taken with Reddy, G. B. *et al.* (*J. Biol. Chem.* (2000) 275, 4566-4570) and Wilson, M. J. *et al.* (US Pat. No. 6310186).

Gopalakrishnan. *et al.* teach preparation of α -crystallin proteins from bovine eye by homogenization in a biochemical buffer (see page 2937), and separating the protein in an affinity chromatography, as applied to item a) of claim 3. Yet, Gopalakrishnan. *et al.* do not expressly teach purification of the crystallin protein using anion exchange chromatography.

Reddy *et al.* teach purification of a mammalian α -crystallin protein *via* Q-Sepharose anion exchange column wherein the protein is eluted with high NaCl followed by the second chromatography (see the left column at page 4566), as applied to item b) to c) of claim 3.

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Wilson, M. J. *et al.* teach purification of biological preparations, especially proteins, that have been produced by recombinant DNA techniques in bacteria using Macrorep High Q column equilibrated with buffer containing Glycine (see abstract and column 7), as applied to item d) of claim 3.

One of ordinary skill in the art would have combined the teachings of Gopalakrishnan. *et al.* together with Reddy *et al.* and Wilson *et al.* to develop a method for purifying bovine α -crystallin protein using anion-exchange column-based chromatography, i.e., the two chromatographic processes – one is Q-Sepharose anion exchange column and the other Macrorep type anion exchange column that offers the material choice for low pressure chromatography applications, and the Macrorep resin exhibits non-specific binding properties differing from those of Sepharose[®] and thus can also be recommended in cases where unsatisfactory results are obtained with Sepharose and vice versa, as suggested by manufacture (BioRad Inc.). When combined, it would furthermore results in the following further advantages: (i) improved method of the first ion exchange chromatography followed by the second anion exchange chromatography, i.e., utilizing Macrorep column in order for reducing the content of contaminants in biological preparations as taught by Wilson *et al.* (see abstract); and (ii) as an alternative procedure, anion exchange chromatography followed by size exclusion chromatography would have been beneficial because the former process requires high salt for elution whereas the latter functions desalting apart from further purification of the protein. Thus, the claimed invention was *prima facie* obvious to make and use at the time it was made.

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Claim 4 is rejected under 35 U.S.C. 103(a) as being obvious over Cobb, B. A. *et al.* (*J. Biol. Chem.* (2000) 275, 6664-6672) taken with Guo, Z. *et al.* (*Bioch. Biophys. Res. Comm.* (2000) 270, 183-189).

Cobb *et al.* teach a method of purifying recombinant α -crystallin proteins which is conjugated to a molecular marker tag for measurement of interaction of the crystallin protein with membrane and other biomolecules (see page 6665). Guo *et al.* teach a 6-His tagged chaperone protein, *i.e.*, hsp25 (note that hsp25 and α -crystallin are closely related in structure and belong to *small heat shock protein* family as they share a consensus sequence at C-terminal region (see the first paragraph, page 183)), and teach constructing hexahistidine-hsp25 fusion, transformation a bacterial strain for expression of the fusion construct, lysis of the host expressing the recombinant protein and purification the protein by pH elution from Nickel NTA resin (see "Materials and Methods" section, page 184). The Guo *et al.* reference together with the Cobb *et al.* reference are applied to items a) to d) of claim 4.

One of ordinary skill in the art would have combined the teachings of Cobb *et al.* together with Guo *et al.* to develop a method for purifying recombinant α -crystallin protein tagged with hexahistidine sequence using nickel NTA resin chromatography thereof. When combined, it would have resulted in the following advantages: (i) not only can be 6-His tagged chaperone protein rapidly purified but also the purified recombinant proteins have biological activity compared to intact protein (see Table 1 data, and the right column, the second paragraph) and are useful for continued investigation of the chaperone function both *in vivo* and *in vitro* taught by Guo *et al.* (see the last paragraph); and (ii) the tagged α -crystallin protein retains

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biological binding, *e.g.* biomembrane binding as taught by Codd *et al.* Thus, the claimed invention was *prima facie* obvious to make and use at the time it was made.

Claim 5 is rejected under 35 U.S.C. 103(a) as being obvious over Gopalakrishnan, S. *et al.* (*Invest. Ophthalmol. Vis. Sci.* (1992) 33, 2936-2941) taken with Wangner, B. J. *et al.* (*Ach. Biochem. Biophys.* (1995) 323, 455-462).

Gopalakrishnan *et al.* teach a method of covalently coupling bovine α -crystallin to a chromatographic resin which is cyanogens bromide activated Sepharose 4B (see "Material" section and page 2937), and a process of binding of the proteins to Sepharose- α -crystallin complex using a blocking solution comprising bovine serum albumin (BSA) and elution of the bound proteins on the affinity column (see the right column, page 2937), as applied to items a) to c) of the claim 5. In addition, Gopalakrishnan *et al.* show that immobilized α -crystallin proteins have biological activity revealed by their ability of subunit-subunit interaction. Yet, Gopalakrishnan *et al.* do not explicitly teach using the resin immobilized α -crystallin for preventing undesired proteolysis during separation of the interest protein.

Wagner *et al.* teach inhibition of proteasome, a complex composed of plurality of protease/peptidases by the purified bovine α -crystallin.

One of ordinary skill in the art would have been motivated by the Wagner *et al.* and the Gopalakrishnan *et al.* teachings as to the resin-coupled α -crystallin retains biological functions including protease inhibitory activity and would have combined the teachings of Wagner *et al.* and Gopalakrishnan *et al.* to make and sue a chromatographic column wherein bovine α -crystallin acting as protease inhibitor is immobilized on solid phase, *i.e.*, the chromatographic

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resins, while the proteins to be separated are in a mobile phase so that proteolysis would have been inhibited or reduced to a large extent during chromatography. When combined, this would have resulted in the advantages: (i) the immobilized α -crystallin protein retains binding specificity compared to "membrane-immobilized" α -crystallin as taught by the Gopalakrishnan *et al.* reference (see the last paragraph of Introduction section, page 2936); and (ii) not only is a single type of protease inhibited but multiple proteases/peptides are inhibited by the coupled α -crystallin proteins so that the protein of choice can be purified in high yield as well as in a soluble and active form as taught by the Wagner *et al.* reference.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Samuel Wei Liu whose telephone number is (703) 306-3483. The examiner can normally be reached from 9:00 a.m. to 5:00 p.m. on weekdays. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dr. Christopher Low, can be reached on 703 308-2923. The fax phone number for the organization where this application or proceeding is assigned is 703 308-4242 or 703 872-9306 (official) or 703 872-9307 (after final). Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 703 305-4700.

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October 28, 2002

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